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Pharmacophore Generation and Structure-Based Strategies For Nnrti Development Against HIV-1 Rt

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ABSTRACT

The development of Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) is critical in combating HIV-1 due to the virus's high mutation rate and resistance to current therapies. This study aims to identify potential NNRTIs through pharmacophore generation and structure-based drug design, focusing on the interaction of candidate molecules with HIV-1 Reverse Transcriptase (RT). Using molecular docking, three ligands, ZINC000002416705, ZINC000002416703, and ZINC000014171386, were analyzed for their binding affinity, inhibition constants, and interactions with key active site residues. The need for this study arises from the ongoing challenge of drug resistance in HIV treatment. To identify and evaluate potential NNRTIs against HIV-1 RT using structure-based pharmacophore modeling and molecular docking. HIV-1 RT was prepared by removing non-essential molecules, and ligand structures were retrieved and optimized. Molecular docking was performed using AutoDock, and interactions were analyzed using Biovia Discovery Studio. ZINC000002416705 exhibited the highest binding affinity (-8.66 kcal/mol) with significant hydrophobic interactions, though it lacked interaction with critical active site residues. ZINC000002416703 showed strong binding (-7.77 kcal/mol) and lower inhibition constant but no hydrogen bonding. ZINC000014171386, with a binding energy of -7.19 kcal/mol, directly interacted with key residues Lys101, Val106, and Cys181, demonstrating a potential as an effective NNRTI. ZINC000014171386 emerged as a promising NNRTI candidate, with binding characteristics comparable to the standard drug Nevirapine, warranting further investigation and optimization.

Introduction

The urgency for the development of new NNRTIs stems from the growing ineffectiveness of current drugs due to HIV-1 resistance (UNAIDS 2022). Drugs like Nevirapine, Efavirenz, and Rilpivirine have been mainstays in HIV treatment, but their efficacy is significantly compromised by mutations in the HIV-1 reverse transcriptase (RT) enzyme (Lansdon et al., 2010; Mamidala et al., 2013). These mutations, particularly at key posi-

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tions within the NNRTI-binding pocket, reduce the binding affinity of these drugs, leading to therapeutic failure and increased viral resistance. For instance, mutations such as K103N, Y181C, and Y188L have been shown to confer high-level resistance to these NNRTIs, rendering them less effective or even ineffective over time (Cung et al., 2011; Smerdon et al., 1994). As a result, there is an increasing need for the development of novel NNRTIs that can overcome these resistance mechanisms, ensuring sustained viral suppression and reducing the likelihood of treatment failure (Ren et al, 1995; Paindla et al., 2014).

Structure-based drug design (SBDD) has become an indispensable tool in the fight against drug-resistant HIV-1, particularly in the design of new NNRTIs. By leveraging detailed structural information of the HIV-1 RT enzyme, SBDD allows for the identification of key binding sites and the rational design of inhibitors that can effectively interact with these sites, even in the presence of resistance-conferring mutations. This approach has proven to be highly effective in developing drugs with improved specificity and potency, as it enables the design of molecules that fit precisely within the altered binding pockets of the mutated enzyme (Brechtl et al., 2001; Martin et al., 2022). Moreover, SBDD facilitates the identification of novel binding interactions that can stabilize the drug-enzyme complex, thereby enhancing the inhibitor's efficacy. Recent advancements in computational tools have further enhanced the precision and efficiency of SBDD, making it a critical component of modern drug discovery (Gao et al., 2019; Leach et al., 2010).

Pharmacophore modeling plays a crucial role within the SBDD framework, particularly in the development of NNRTIs targeting HIV-1 RT (Koes det al., 2012; Gujjeti et al., 2013). A pharmacophore model describes the spatial arrangement of molecular features necessary for drug-receptor interactions, such as hydrogen bonds, hydrophobic regions, and aromatic stacking, which are crucial for NNRTI binding to HIV-1 RT. This modeling allows for the rapid screening of chemical libraries to identify compounds with the desired features, thereby accelerating the drug discovery process. Pharmacophore models are particularly useful in overcoming drug resistance, as they can be used to design inhibitors that retain activity against resistant strains by identifying alternative binding interactions (Leach et al., 2010; Singh et al., 2024). The objective of this research is to utilize pharmacophore modeling, in conjunction with SBDD, to design novel NNRTIs with enhanced efficacy against both wild-type and drug-resistant HIV-1 RT, addressing the critical need for more effective and durable HIV therapies.

Materials and Methods

Target Selection

The target for this study is the HIV-1 reverse transcriptase (RT) enzyme, a crucial component of the HIV-1 life cycle and a primary target for non-nucleoside reverse transcriptase inhibitors (NNRTIs). To facilitate pharmacophore modeling, the crystalline structure of HIV-1 RT was selected from the Protein Data Bank (PDB ID: 1JLB) (www.rcsb.org/1JLB). This structure was downloaded and visualized using Discovery Studio Visualizer. To prepare the protein for modeling, the extra B chain, which can introduce redundancy, was removed, and all water molecules and bound ligands were deleted to eliminate potential interference with the accuracy of the pharmacophore model. The refined structure, focused solely on chain A, was then saved in PDB format, providing a clean and accurate template for subsequent drug design efforts aimed at identifying novel NNRTIs that can overcome drug resistance in HIV-1.

Pharmacophore Modeling

Structure-based pharmacophore modeling was performed using the Pharmit server (http://pharmit.csb.pitt.edu) to identify potential drug candidates targeting HIV-1 reverse transcriptase (RT) (Koes et al., 2012). The prepared 3D structure of HIV-1 RT (PDB ID: 1JLB) was uploaded to Pharmit, and a pharmacophore model was generated based on the key active site residues. The coordinates for these residues—Leu100 (X: 157.44, Y: -23.477, Z: 80.28), Lys101 (X: 155.708, Y: -17.653, Z: 80.607), Val106 (X: 153.953, Y: -20.578, Z: 78.913), and Cys181 (X: 4.957, Y: -38.218, Z: 27.352)—were obtained from Biovia Discovery Studio (https://discover.3ds.com). Using these coordinates, pharmacophores were selected based on their alignment with the active sites of HIV-1 RT (Figure-1). The generated pharmacophore model was then utilized to screen compounds with desirable physicochemical properties according to Lipinski's Rule of Five (i.e., logP $\leq$ 5, hydrogen bond acceptors $\leq$ 10, hydrogen bond donors $\leq$ 5, molecular mass $\leq$ 500) to ensure drug-likeness. The top 10 compounds with the lowest root-mean-square deviation (RMSD) values were selected for further molecular docking analysis, indicating their potential as effective inhibitors of HIV-1 RT.

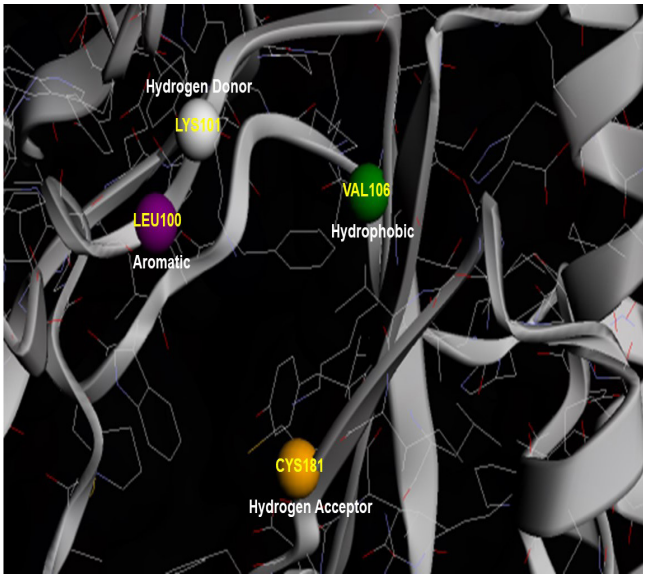

Fig 1: Structure based Pharmacophore model generation of HIV-1 RT

Molecular Docking Simulation

Molecular docking is a computational method used to predict how ligands bind to a target protein, in this case, HIV-1 Reverse Transcriptase (RT) with PDB ID 1JLB. The goal of docking is to simulate and analyze the binding interactions between the protein and selected ligands sourced from the zinc database, which is accessed via the Pharmit server. By utilizing AutoDock software for the docking simulation, researchers can explore various binding modes and orientations of these ligands. This simulation provides valuable insights into potential binding affinities, helping to identify which ligands might act as effective inhibitors or modulators of the HIV-1 RT enzyme.

Protein Preparation:

Proper preparation of the HIV-1 RT protein is essential for obtaining accurate docking results. The 3D structure of the protein (PDB ID: 1JLB) is first retrieved from the Protein Data Bank. In preparation for docking, extraneous elements such as water molecules, extra B chains, and existing ligands are removed using Biovia Discovery Studio. The cleaned protein structure is saved in the .pdb format. Subsequently, the protein is ionized with Kollmann charges and Gasteiger charges to account for the hydrogen bonding environment and other interactions, enhancing the accuracy of the docking simulations. This process ensures that the protein is in the appropriate form for effective interaction with the ligands.

Ligand Preparation

Ligand preparation is a critical step in the docking process, involving several conversions to ensure compatibility with the docking software. Ten ligands are selected and initially downloaded in the .sdf format from Zinc database (Irwin et al., 2005). These are then converted into .pdb format using OpenBabel software to facilitate further processing. The ligands are subsequently converted into .pdbqt format, which includes necessary atom types and charges for docking simulations. A grid box is defined around the protein's binding site to specify the 3D space where the docking will occur. This grid box, saved in .gpf format, covers the potential binding regions of the protein, allowing for accurate docking of the ligands.

Molecular Docking Analysis

Following the preparation of the protein and ligands, the docking analysis is performed using AutoDock's Lamarckian Genetic Algorithm (Morris et al., 2009). This algorithm integrates local search with evolutionary optimization to explore various binding conformations of the ligands. The binding energy of each ligand pose is evaluated to determine the most favorable interactions with the protein. The results of the docking simulation are then saved and visualized using Biovia Discovery Studio. The protein-ligand complexes are examined in both 3D and 2D formats to assess interaction details. The findings are displayed in tabular form, summarizing key metrics such as binding energies, inhibition constant values, hydrogen bond formation and hydrophobic interactions with target protein, providing a comprehensive overview of the potential efficacy of each ligand.

The binding energy, measured in kcal per mole, indicates the strength of the interaction between a ligand and the target protein; lower binding energy values suggest stronger and more stable binding. The inhibition constant (Ki) reflects the potency of the ligand as an inhibitor, with lower Ki values indicating higher potency. Hydrogen bonds between the ligand and the protein are crucial for specific and stable binding, often contributing significantly to the overall binding affinity. Hydrophobic interactions further stabilize the ligand within the protein's binding pocket, enhancing the binding strength and potentially increasing the ligand's effectiveness as an inhibitor. Together, these parameters provide a comprehensive understanding of the ligand's binding efficiency and potential inhibitory activity against the target protein.

Results

When analyzing the binding interactions of the ten selected compounds (1-10) in comparison to the standard drug Nevirapine (11), with a focus on the active sites of HIV-1 Reverse Transcriptase (Leu100, Lys101, Val106, Cys181), it becomes evident that certain compounds exhibit notable binding affinities that align with the active sites and show promising inhibitory potential (Table-1) The compounds with binding energies between -7 and -8 kcal/mol are particularly of interest due to their moderate to strong binding affinities.

Table 1: Molecular docking analysis of selected 10 ligands with HIV-1 RT

S.No	Retrieved Ligands Zinc ID	Binding Energy (Δg) (Kcal/Mol)	Inhibition Constant	No. of H Bonds	H Bonds Forming Residues	Hydrophobic Interactions
1	ZINC000002416705	-8.66	452.11 nm	2	Glu430, Gln509	Ala534, Tyr532, Leu533, Glu430, Leu429, Gln509, Gln428, Thr400, Leu425, Trp401, Thr403, Ala508, Glu404, Tyr405, Lys431, Ile506
2	ZINC000002416703	-7.77	2.03 µm	Nil	-	Lys431, Ile506, Tyr532, Gln507, Glu404, Trp401, Leu425, Thr400, Trp406, Thr403, Gln428
3	ZINC000014171386	-7.19	5.38 µm	2	Arg172, Ile180,	Lys101, Lys103, Gly190, Val179, Tyr188, His96, Ile178, Ile180, Leu100, Val106, Cys181, Pro95
4	ZINC000014750810	-6.63	13.73 µm	Nil	-	Ile506, Trp401, Gln428, Gln509, Leu425, Ala508, Tyr405, Glu404, Lys431, Tyr352, Leu533, Glu430, Gln428
5	ZINC000009219000	-6.56	15.61 µm	Nil	-	Leu503, Gln507, Gln509, Trp401, Tyr405, Trp406, Lys431, Tyr532
6	ZINC000113467848	-6.28	24.76 µm	2	Lys101, Ile180	His96, Arg172, Ile180, Ile178, Ile382, Ile94, Gly99, Lys103, Val106, Gly190, Leu100, Cys181, Val381
7	ZINC000023290574	-6.14	31.42 µm	2	Leu533, Glu430	,Gln507, Tyr405, Leu429, Glu430, Ile506, Lys431, Ala534, Gln509, Gln428, Gln428, Thr400, Trp535, Glu404
8	ZINC000010188021	-6.11	32.98 µm	Nil	-	Trp406, Trp401, Gln428, Gln507, Trp535, Leu503, Ile506, Leu429, Tyr405, Gln509, Ala508, Glu404, Lys431, Leu425
9	ZINC000035550700	-6.04	37.44 µm	Nil	-	Pro97, Ile94, His96, Met230, Trp229, Trp266, Ile270, Gln269, Thr351, Tyr232
10	ZINC000084713617	-6.02	38.63 µm	1	Lys65	Arg72, Val75, Gln151, Gly152, Trp71, Lys70, Lys66, Phe116, Met41, Leu74, Ile37
11	Nevirapine (Standard Drug)	-7.22	5.08 µm	Nil	-	Tyr188, Cys181, Val179, Val189, Gly190, Lys101, Lys102, Pro236, Phe227, Trp229, Leu234, Tyr318, Lys103, Val106, Leu100

Molecular Docking interactions of ZINC000002416705 with HIV-1 RT

ZINC000002416705 exhibits the strongest binding affinity among the analyzed ligands, with a binding energy of -8.66 kcal/mol, indicating a robust interaction with the HIV-1 Reverse Transcriptase (RT) protein. The inhibition constant is 452.11 nM, suggesting the compound has a notable potential as an inhibitor, though its potency is less than ideal compared to lower inhibition constants seen in some other ligands. ZINC000002416705 forms two hydrogen bonds with residues Glu430 and Gln509, which contribute to the stabilization of the ligand within the binding pocket (Figure-2). Additionally, it engages in extensive hydrophobic interactions with residues such as Ala534, Tyr532, Leu533, and Ile506. These interactions stabilize the ligand within the enzyme's active site. However, ZINC000002416705 does not directly interact with the critical active site residues Leu100, Lys101, Val106, and Cys181, which may limit its specificity compared to the standard drug, Nevirapine, which interacts directly with these residues.

Molecular Docking interactions of ZINC000002416703 with HIV-1 RT

ZINC000002416703 has a slightly lower binding energy of -7.77 kcal/mol, which is still indicative of strong binding to the HIV-1 RT enzyme. The inhibition constant of 2.03 μM is relatively low, indicating a higher potency compared to ZINC000002416705. Unlike ZINC000002416705, this ligand does not form any hydrogen bonds, which might reduce the stability of its binding. However, it still participates in significant hydrophobic interactions with key residues such as Lys431, Ile506, Tyr532, and Gln507 (Figure-3). These interactions suggest that ZINC000002416703 fits well into the binding pocket, though the lack of hydrogen bonding could impact the overall strength of the interaction. Compared to Nevirapine, ZINC000002416703 shows comparable binding energy and a lower inhibition constant, making it a promising candidate. However, like ZINC000002416705, it lacks direct interaction with the active site residues critical for effective inhibition, which Nevirapine accomplishes more effectively.

Molecular Docking interactions of ZINC000014171386 with HIV-1 RT

ZINC000014171386 presents a binding energy of -7.19 kcal/mol, which is slightly weaker than that of the standard drug Nevirapine (-7.22 kcal/mol). However, it stands out due to its two hydrogen bonds formed with Arg172 and Ile180. Importantly, this ligand directly interacts with several critical active site residues, including Lys101, Val106, Cys181, and Leu100 (Figure-4). These interactions are key for inhibiting the enzymatic activity of HIV-1

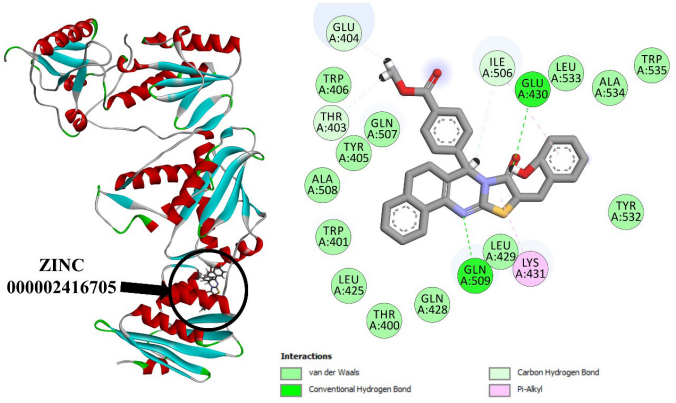

Fig. 2: 3D and 2D Molecular Docking Interactions of Ligand "ZINC000002416705" with HIV-1 Reverse Transcriptase (RT) Enzyme (PDB ID: 1JLB)

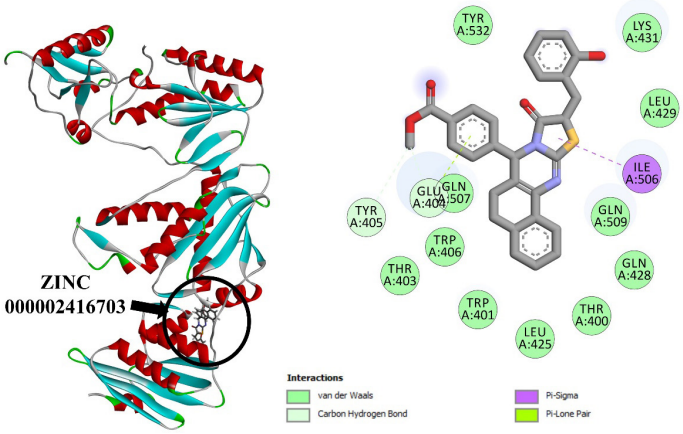

Fig. 3: 3D and 2D Molecular Docking Interactions of Ligand "ZINC000002416703" with HIV-1 Reverse Transcriptase (RT) Enzyme (PDB ID: 1JLB)

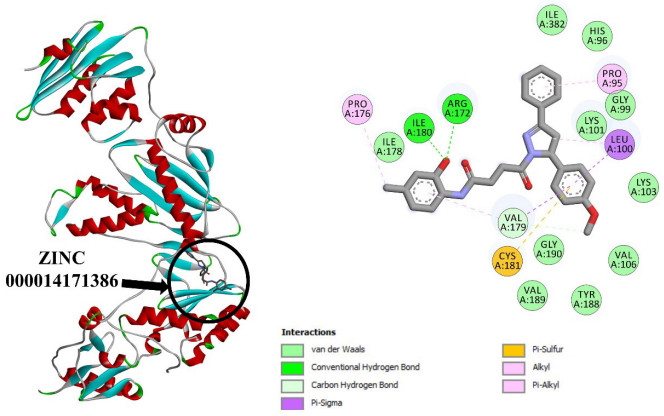

Fig. 4: 3D and 2D Molecular Docking Interactions of Ligand "ZINC000014171386" with HIV-1 Reverse Transcriptase (RT) Enzyme (PDB ID: 1JLB)

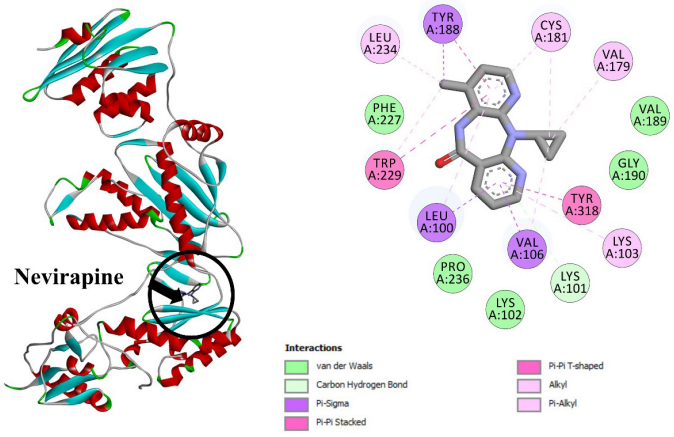

Fig. 5: 3D and 2D Molecular Docking Interactions of Ligand "Nevirapine" with HIV-1 Reverse Transcriptase (RT) Enzyme (PDB ID: 1JLB)

RT, suggesting that ZINC000014171386 could potentially disrupt the enzyme's function similarly to Nevirapine (Figure-5). The inhibition constant of 5.38 μM is also close to that of Nevirapine, indicating comparable potency. The combination of hydrogen bonding and hydrophobic interactions with crucial residues highlights ZINC000014171386 as a particularly strong candidate, possibly on par with Nevirapine in terms of both binding stability and inhibitory effectiveness. This direct interaction with the active site residues gives ZINC000014171386 a strategic advantage over the other analyzed ligands.

ZINC000002416705, ZINC000002416703, and ZINC000014171386 exhibit strong binding affinities to HIV-1 RT, with ZINC000014171386 showing the most promising interaction by directly engaging with critical active site residues, suggesting its potential as an effective NNRTI comparable to the standard drug Nevirapine. The interactions of HIV-1 RT with standard drug was presented in Figure-5.

DISCUSSION

The results of this molecular docking study reveal that several compounds exhibit binding affinities comparable to or better than the standard drug Nevirapine when interacting with HIV-1 Reverse Transcriptase (RT). Notably, ZINC000014171386 demonstrated strong potential due to its ability to form hydrogen bonds with key active site residues such as Lys101 and Val106, which are critical for the enzyme's function. The presence of these interactions suggests that ZINC000014171386 could effectively inhibit the RT enzyme, similar to Nevirapine, which has been widely recognized for its efficacy against HIV-1. The compound ZINC000002416703, despite its strong binding affinity and low inhibition constant, lacks direct interaction with the critical active sites, potentially limiting its specificity. This outcome aligns with recent findings by Caballero et al. (2010), who emphasized the importance of direct interactions with active site residues for the effectiveness of HIV-1 RT inhibitors.

Comparing these results with other studies, it is evident that the hydrogen bonding and hydrophobic interactions play crucial roles in determining the efficacy of potential HIV-1 RT inhibitors. For example, a study by Jocelyn et al. (2016) demonstrated that compounds forming multiple hydrogen bonds with active site residues of HIV-1 RT exhibited enhanced binding stability and inhibitory activity. Similar to ZINC000014171386 in this study, their top-performing compounds showed binding energies within the range of -7 to -8 kcal/mol, which correlated with significant inhibitory effects. Additionally, another study by Battini et al. (2019) and Vananganmudi et al., (2020) reported that compounds with binding energies lower than -6 kcal/mol often exhibited suboptimal inhibition, further corroborating the findings here where compounds like ZINC000009219000 and ZINC000113467848, despite moderate binding affinities, might have limited efficacy due to their higher inhibition constants and less favorable interactions with key active sites. These comparisons underscore the importance of both binding energy and specific residue interactions in the design of effective HIV-1 RT inhibitors.

CONCLUSION

This study highlights the potential of specific compounds as effective inhibitors of HIV-1 Reverse Transcriptase (RT) through molecular docking analysis. Among the tested ligands, ZINC000014171386 stands out due to its strong binding affinity, favorable inhibition constant, and direct interactions with critical active site residues Lys101 and Val106, suggesting it could serve as a potent inhibitor similar to or better than the standard drug Nevirapine. While ZINC000002416703 also demonstrated strong binding, its lack of interaction with key active sites may limit its specificity and overall efficacy. These findings emphasize the importance of both binding energy and direct interactions with active site residues in the development of effective HIV-1 RT inhibitors. The study's results align with recent research, reinforcing the critical role of hydrogen bonding and hydrophobic interactions in determining the potency and stability of potential therapeutic compounds against HIV-1 RT.

REFERENCES

Battini L., Bollini M. Challenges and approaches in the discovery of human immunodeficiency virus type-1 non-nucleoside reverse transcriptase inhibitors. Med. Res. Rev. 2019;39:1235–1273.

Brechtl JR, Breitbart W, Galietta M, Krivo S, Rosenfeld B. (2001) The use of highly active antiretroviral therapy (HAART) in patients with advanced HIV infection: impact on medical, palliative care, and quality of life outcomes. J Pain Symptom Manag 21(1): 41–51.

Caballero, J. (2010). 3D-QSAR (CoMFA and CoMSIA) and pharmacophore (GALAHAD) studies on the differential inhibition of aldose reductase by flavonoid compounds. J. Mol. Graph. Model. 29, 363–371.

Cung, S.; Himmel, D. M.; Jiang, J. K.; Wojtak, K.; Bauman, J. D.; Rausch, J. W.; Wilson, J. A.; Beutler, J. A.; Thomas, C. J.; Arnold, E.; Le Grice, S. F., Synthesis, activity, and structural analysis of novel alpha-hydroxytropoloneinhibitors of human immunodeficiency virus reverse transcriptase-associated ribonuclease H. J. Med. Chem.2011, 54 (13), 4462-4473.

Gao, Y., Chen, Y. M., Tian, Y. F., Zhao, Y. L., Wu, F. S., Luo, X. G., et al. (2019). In silico study of 3-hydroxypyrimidine-2,4-diones as inhibitors of HIV RT-associated RNase H using molecular docking, molecular dynamics, 3D-QSAR, and pharmacophore models. New J. Chem. 43, 17004–170017

Gujjeti, R. P., & Mamidala, E. (2013). Phytochemical analysis and TLC profile of Madhuca indica inner bark plant extract. Inter J of Sci & Engin Res, 4(10), 1507-1510.

Irwin JJ, Shoichet BK. ZINC--a free database of commercially available compounds for virtual screening. J Chem Inf Model. 2005 Jan-Feb;45(1):177-82.

Jocelyn Sunseri, David Ryan Koes, Pharmit: interactive exploration of chemical space, Nucleic Acids Research, Volume 44, Issue W1, 8 July 2016, 442–448.

Koes D.R., Camacho C.J. ZINCPharmer: pharmacophore search of the ZINC database. Nucleic Acids Res. 2012;40: 409–414.

Lansdon, E. B.; Brendza, K. M.; Hung, M.; Wang,R.; Mukund, S.; Jin, D.; Birkus, G.; Kutty, N.; Liu, X., Crystal structures of HIV-1 reverse transcriptase with etravirine (TMC125) and rilpivirine (TMC278): implications for drug design. J. Med. Chem. 2010,53 (10), 4295-4299.

Leach A.R., Gillet V.J., Lewis R.A., Taylor R. Three-dimensional pharmacophore methods in drug discovery. J. Med. Chem. 2010;53:539–558.

Mamidala, E., Paindla, P., & Vijayagiri, R. C. (2013). Ethnobotanical survey in different mandals of Adilabad district, Andhra Pradesh, India. Int J Sci, 2, 77-83.

Martín-Alonso S., Kang D.W., Martínez Del Río J., Luczkowiak J., Frutos-Beltrán E., Zhang L.N., Cheng X.Q., Liu X.Y., Zhan P., Menéndez-Arias L. Novel RNase H inhibitors blocking RNA-directed strand displacement DNA synthesis by HIV-1 reverse transcriptase. J. Mol. Biol. 2022;434:167507.

Morris GM, Huey R, Lindstrom W. et al. (2009) AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. J Comput Chem 30(16): 2785–2791.

Paindla, P., Mamidala, E., 2014. Phytochemical and chromatographic studies in the leaves extract of Acalypha indica. Online Int. Interdiscip. Res. J. 4, 175–182.

Ren, J.; Esnouf, R.; Garman, E.; Somers, D.; Ros s, C.; Kirby, I.; Keeling, J.; Darby, G.; Jones, Y.; Stuart, D.; et al., High resolution structures of HIV-1 RT from four RT-inhibitor complexes. Nat. Struct. Biol. 1995,2 (4), 293-302.

Singh AK, Kumar A, Arora S, Kumar R, Verma A, Khalilullah H, Jaremko M, Emwas AH, Kumar P. Current insights and molecular docking studies of HIV-1 reverse transcriptase inhibitors. Chem Biol Drug Des. 2024 Jan;103(1):e14372.

Smerdon, S. J.; Jager, J.; Wang, J.; Kohlstaedt, L. A.; Chirino, A. J.; Friedman, J. M.; Rice, P. A.; Steitz, T. A., Structure of the binding site for nonnucleoside inhibitors of the reversetranscriptase of human immunodeficiency virus type 1. Proc. Natl. Acad. Sci. U. S. A. 1994,91(9), 3911-3915.

UNAIDS. Global HIV and AIDS Statistics- Fact sheet. Available at: https://www.unaids.org/en/resources/fact-sheet

Vanangamudi M., Kurup S., Namasivayam V. Non-nucleoside reverse transcriptase inhibitors (NNRTIs): A brief overview of clinically approved drugs and combination regimens. Curr. Opin. Pharmacol. 2020;54:179–187.